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ORIGINAL PAPER

Particle size reduction along the digestive tract of fat sand rats (*Psammomys obesus*) fed four chenopods

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Abstract

It is generally accepted that microbial digestion contributes little to digesta particle size reduction in herbivores, and that faecal particle size reflects mainly chewing efficiency, and may vary with diet. Nevertheless, a decrease in mean particle size (MPS) along the gastrointestinal tract (GIT) has been reported, especially in hindgut fermenters. However, to what degree the very fine particle fraction (non-food origin, especially microbes) affects MPS is unclear. Fat sand rats (*Psammomys obesus*, diurnal herbivores, $n = 23$, $175 \pm$ sd 24 g) consumed one of four chenopods (natural dietary items in the wild) for 30 days. Digestibility was related negatively to dietary fibre content. We determined digesta MPS in the forestomach, glandular stomach, small intestine, caecum, colon and faeces by wet sieving, including (MPS_{fines}) or excluding ($MPS_{nofines}$) particles < 0.25 mm. The proportions of fines were higher and of MPS_{fines} were correspondingly lower in GIT sections that harbour microbes (forestomach, hindgut), whereas $MPS_{nofines}$ did not differ between forestomach and glandular stomach. However, $MPS_{nofines}$ decreased along the GIT, indicating MPS reduction due to digestive (enzymatic and microbial) processes. The four different diets led to different MPS, but the magnitude of MPS reduction in the GIT was not correlated with dietary fibre fractions or dry matter digestibility. These results indicate that within a species, MPS cannot be used as a proxy for diet quality or digestibility, and raise the hypothesis that MPS reduction along the GIT may be more pronounced in smaller than in larger mammalian terrestrial herbivores, possibly due to the fine initial particles produced by chewing in small species.

Keywords Mean particle size · Faecal particle size · Digestive tract · *Psammomys obesus* · Chenopod · Chewing efficiency

Introduction

Smaller food particles can be digested at a higher rate by microbes than larger particles (Bjorndal et al. 1990; Hummel et al. 2020) and, therefore, size reduction of ingested food is beneficial for herbivores. Several stages are involved in the reduction of particle size, including chewing, gastric acid-induced maceration, solubilization of nutrients from disintegrated plant cells, and microbial fermentation itself. Particle size reduction is considered mainly a function of chewing, while microbial fermentation and other digestive processes have only a minor effect, at least in large terrestrial herbivores (Poppi et al. 1980a; Murphy and Nicoletti 1984; McLeod and Minson 1988; Spalinger and Robbins 1992). This was clearly illustrated by the recovery of intact skeletal leaf structures from the faeces of folivorous, non-chewing reptiles (Fritz et al. 2010). Consequently, little further reduction in particle size beyond the stomach is expected (Poppi et al. 1980b; Lechner-Doll and von Engelhard 1989) and,

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as a result, faecal particle size has been used as a measure of 'chewing efficiency' in terrestrial herbivores (Fritz et al. 2009). By contrast, seagrass, which does not need lignified rigid structures to cope with gravity, was reduced substantially in particle size along the intestinal tract of an aquatic herbivore, the dugong (*Dugong dugon*) (Lanyon and Sanson 2006).

Nevertheless, this does not mean that digestion, and in particular microbial fermentation, does not contribute to particle size reduction in terrestrial herbivores (see Krämer et al. 2013)—it simply means that its contribution seems to be of much less importance. One could predict that the contribution of digestion to particle size reduction is a function of initial size itself, in particular at the measurement resolution offered by sieve analyses, which does not directly record shape changes but only the capacity of particles to pass certain sieve pores. Microbial action should disrupt the integrity of a small particle more easily than that of a large particle. If this prediction is true, one would expect little relevant change in particle size along the digestive tract in large herbivore species. This is evident, for example, in horses, where the mean particle size in faeces is similar to the mean particle size in the stomach (Clauss, pers. obs.), and in ruminants, where the particle size in faeces is similar to the size of material passing out of the reticulo-rumen (Poppi et al. 1980a; Lechner-Doll and von Engelhardt 1989; Naumova et al. 2012). By contrast, smaller herbivorous species, such as rodents, achieve much finer particles by chewing, which opens the possibility to detect a more distinct effect of digestion and of microbial action on particle size. Thus, a decrease in particle size along the digestive tract, similar to that described for the dugong, would be expected in small herbivores. Such a decrease has been reported in several species, for example koalas (*Phascolarctos cinereus*) (Lanyon and Sanson 1986), field voles (*Microtus agrestis*) (Zharova et al. 2005), a sloth (*Choloepus didactylus*), pygmy hippos (*Choeropsis liberiensis*) and wallabies (*Macropus rufogriseus*) (Schwarm et al. 2013), hares (*Lepus europaeus, L. timidus*) (Naumova et al. 2015a), mole voles (*Ellobius talpinus*) (Naumova et al. 2018), and maras (*Dolichotis patagonum*) (Clauss et al. 2019).

In these kinds of experimental assessments, several aspects need to be controlled. Evidently, a consistent diet should be fed to the animals for a period corresponding to at least two times the digesta retention time, to ensure that the digesta represents the same diet at all sites in the gastrointestinal tract (GIT)—a condition not necessarily met for the sloth and pygmy hippos in Schwarm et al. (2013). Additional mastication comminution by merycism, which may occur both in koalas (Logan 2003) and macropods (Vendl et al. 2017), and the potential effect of a colonic separation mechanism (CSM) (Bjornhag and Snipes 1999) should be considered.

The CSM separates very fine particles from the digesta in the colon and directs them into the caecum (Bjornhag and Snipes 1999; Cork et al. 1999). As microbes represent very fine particles, the CSM prevents the elimination of microbes with the regular or 'hard' faeces. Instead, the microbes are retained in the caecum and eliminated separately in 'soft faeces' or 'caecotrophs' that can be re-ingested by the animal—a process termed 'coprophagy' or 'caecotrophy'. This mechanism facilitates the use of fibrous diets in small herbivores (Foley and Cork 1992).

Hence, the CSM may accumulate fine digesta particles in the caecum (Lanyon and Sanson 1986; Vispo and Hume 1995; Naumova et al. 2015a, b, 2017; Clauss et al. 2019). Comparing stomach and caecum contents alone, therefore, cannot differentiate between the size-reducing effect of microbial digestion and the selective accumulation of fine particles due to the action of the CSM in ingested plants. Rather, data on colon and rectum contents need to be included. Moreover, depending on the time lag between sampling and coprophagy, variable results may be achieved (Naumova et al. 2015a, b). An animal that has just performed corprophagy will have more fine particles, including microbes, in its stomach than an animal that has just ingested vegetation.

Most importantly, a methodological constraint of the standard quantification of mean particle size must be considered. The particle size of digesta or faeces is typically quantified by wet sieving over a cascade of sieves of decreasing pore size (Udén and Van Soest 1982; Fritz et al. 2012; Naumova et al. 2017). In these analyses, a question that arises is what to do with the material that passes the finest sieve. This material can be retrieved by either centrifugation (e.g., Meyer et al. 1986) or by filter paper (e.g. Naumova et al. 2017) or estimated by comparing the dry mass of retained particles to the dry mass of material that is sieved (e.g., Matsuda et al. 2014). Microbes are present in digesta and faeces and, due to their small size (< 0.01 mm, Frobisher et al. 1974), are typically retrieved in the fraction of very fine particles that passed the finest sieve. If this fraction is included in the calculation of mean particle size, it will inadvertently lead to an overestimation of the true size reduction effect, as it includes an unknown mix of digesta particles and microbes. In theory, this fraction should be distinctively larger in proportion at sites of microbial growth, such as the caecum and colon in hindgut fermenters; this was evident in many of the studies cited above.

Finally, particle size reduction by chewing and digestion in herbivores does not only vary with species and body size (Udén and Van Soest 1982; Fritz et al. 2009; Jalali et al. 2015; Naumova et al. 2017), but also with season (Nygren et al. 2001) and hence diet and diet quality (Renecker and Hudson 1990; Hummel et al. 2008). This has been demonstrated especially in a variety of large domestic herbivores

(Jalali et al. 2012a, b, 2015; Kljak et al. 2019). Interestingly, the association with digestibility need not be intuitive: contrary to what was expected, Jalali et al. (2012a) found that more digestible forages resulted in larger faecal particles across sheep, goats and llamas.

Our study aimed at examining changes in particle size along the digestive tract in a model organism of small body size, the fat sand rat (*Psammomys obesus*; Gerbillinae) fed consistently one of four different natural diets. Fat sand rats are widely distributed in the Saharo-Arabian deserts where they inhabit *wadis* (ephemeral riverbeds) and sodic areas that support halophytic vegetation (Mendelssohn and Yom-Tov 1987; Nowak and Paradiso 1983). They are unusual among Gerbillinae in that they are diurnal and wholly herbivorous (Daly and Daly 1973), while other gerbillid species are nocturnal and primarily granivorous (Bar et al. 1984). As adults, fat sand rats live solitarily, and each individual inhabits a complex burrow system with several openings (Orr 1972). They are active above ground all year (Ilan and Yom-Tov 1990). In Israel, fat sand rats inhabit arid areas of the Negev and Judean deserts and the Arava (part of the Rift Valley). They feed on one type of halophytic vegetation, belonging to Chenopodiacae, and the burrow is usually at the base of the plant.

In contrast to most herbivorous small mammals, fat sand rats can thrive while consuming only one chenopod species (Degen 1988; Kam and Degen 1989; Degen et al. 2000). Chenopods are low in organic matter and energy yield and high in inorganic matter, mainly sodium, potassium and chloride, and, because of these negative characteristics, are often avoided by other herbivores. Fat sand rats possess the typical fishbone folds of the Kerckring relief in the mucosa of their proximal colon, the morphological correlate of the colonic separation mechanism. The folds in the fat sand rat are more developed than in other gerbils (Naumova et al. 2011; 2019), but less developed than in voles (Naumova et al. 2018). Fat sand rats practise coprophagy extensively (Khokhlova et al. 2005). Like in many muroid rodents, the forestomach is separated from the glandular stomach by a bordering fold (Naumova et al. 2019). This forestomach typically harbours a microbiome, although its functional relevance remains unclear (reviewed e.g. in Langer and Clauss 2018).

Based on the considerations outlined above, we made the following predictions:

1. digesta particle size is reduced along the GIT of fat sand rats;
2. the very small particle fraction is higher at sites of microbial activity (forestomach, hindgut) than in the glandular stomach and small intestine;
3. both very small and small digesta particles are selectively retained in the caecum; and,
4. particle size, and particle size reduction along the GIT, differ among the four different diets, possibly related to fibre content or digestibility.

Materials and methods

We used 24 adult *Psammamoys obesus* that were bred in the laboratory. They were approximately 12 months of age, were raised at 25 °C with a photoperiod of 12L:12D and were offered only chenopods with no drinking water from birth. The animals were maintained in individual metabolic cages (20 × 10 × 10 cm) with wire-meshed floors allowing collection of total faeces. They were divided randomly into four groups of six animals each, and were offered the green parts of one of four chenopods (collected fresh each day): (1) *Atriplex halimus*; (2) *Suaeda monoica*; (3) *Salsola tetrandra*; and (4) *Anabasis articulata* for ad libitum consumption. Composition of the chenopods is presented in Table 1. No water was offered as the preformed and metabolic water were sufficient. The animals received the feed for 30 days, and chenopod intakes and faeces produced were measured in the last four days before the animals were sacrificed by decapitation in the evening of the following day. The choice of time was only due to logistics involved in terminating the experiments for all animals at the same time; in previous observations, fat sand rats did not show a particular propensity for coprophagy at a specific time of day (Khokhlova et al. 2005). The animals were dissected and the complete contents of the forestomach, glandular stomach, small intestine, caecum, proximal colon and distal colon were collected by everting the respective section of the gastrointestinal tract into a petri dish, and analyzed immediately. The data from one *Psammomys obesus* consuming *Suaeda monoica* was inadvertently misplaced and so the sample size for this diet is five. Additionally, in one of these five animals, faecal material was not sufficient for sieve analysis, reducing the sample size of this group to 4 for all analyses that included faeces. Animal body masses were recorded once, at the end of the experiment. Relative food intake was expressed on the basis of $kg^{0.67}$, following Müller et al. (2013) for small herbivorous mammals.

Daily samples of feed and pooled total faeces were oven dried at 70 °C until constant mass to determine dry matter content. Apparent dry matter digestibility, expressed as a proportion of intake, was calculated by the difference between dry matter intake and dry matter output of faeces. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined in feed samples by standard procedures (Goering and van Soest 1970), using separate samples for NDF and ADF, with the Fibertec system M6 (Tecator, Hoganas, Sweden). Nitrogen content was determined by the Kjeldahl method

Table 1 Chemical composition (mean ± sd) of the four chenopods and body mass, intake and apparent digestibility in *Psammomys obesus*

	Atriplex halimus	*Suaeda monoica*	*Salsola tetrandra*	*Anabasis articulata*
Diet composition				
Dry matter content (% fresh matter)	23.9	20.4	31.8	34.8
Gross energy (kJ/g DM)	13.7 ± 0.2	13.4 ± 0.6	17.8 ± 0.3	16.5 ± 0.3
Ash (% DM)	33.0 ± 0.9	32.7 ± 1.5	18.6 ± 0.9	16.1 ± 0.5
Crude protein (% DM)	20.5 ± 0.7	26.8 ± 1.0	30.8 ± 2.0	22.8 ± 0.2
Neutral detergent fibre (% DM)	34.3 ± 0.8	33.1 ± 2.5	31.2 ± 1.1	37.5 ± 0.6
Acid detergent fibre (% DM)	19.0 ± 0.6	11.1 ± 0.8	12.4 ± 0.4	23.3 ± 0.6
Acid detergent lignin (% DM)	16.1 ± 1.1	5.6 ± 0.4	7.3 ± 0.3	5.5 ± 0.1
Animals				
n	6	5[1]	6	6
Body mass (g)	163.9 ± 18.87	195.0 ± 30.38	177.7 ± 20.62	165.6 ± 19.52
Dry matter intake (g/d)	13.0 ± 1.22[a]	12.5 ± 1.43[a]	12.4 ± 1.16[a]	9.4 ± 1.69[b]
Relative dry matter intake (g $kg^{-0.67}$ d^{-1})	44.0 ± 5.69[a]	37.6 ± 4.96[ab]	39.5 ± 4.14[ab]	31.5 ± 5.05[b]
Relative digestible dry matter intake (g $kg^{-0.67}$ d^{-1})	28.7 ± 3.61[a]	26.1 ± 2.97[a]	27.8 ± 2.96[a]	18.6 ± 3.14[b]
Apparent dry matter digestibility (%)	63.2 ± 2.67[a]	69.7 ± 2.01[b]	70.5 ± 1.25[b]	59.1 ± 3.26[c]
MPS_{fines} reduction (mm)	0.30 ± 0.16	0.20 ± 0.15	0.13 ± 0.06	0.22 ± 0.11
MPS_{fines} reduction (%)	43.8 ± 16.9	28.8 ± 20.4	29.5 ± 17.4	40.2 ± 13.9
$MPS_{nofines}$ reduction (mm)	0.22 ± 0.14	0.19 ± 0.13	0.08 ± 0.08	0.11 ± 0.06
$MPS_{nofines}$ reduction (%)	26.9 ± 15.7	23.6 ± 16.0	16.0 ± 14.6	15.4 ± 5.7

[1]For one animal of this group, insufficient faecal material for sieve analysis led to exclusion of all measurements related to faecal particle size
Means within a row with different superscripts differ from each other, $P < 0.05$

(AOAC, 1990), and crude protein was calculated as Kjeldahl $N \times 6.25$. Energy content of the dry matter of the chenopods was measured with a ballistic bomb calorimeter (Gallenkamp, model CBB-370), using benzoic acid as a standard (26,453 J/g, BCS-CRM no.190n, Bureau of Analyzed Samples, Bristol, UK).

The digesta and faeces were separated into four fractions by rinsing them under tap water over a cascade of soil sieves (Vibrotechnik, C 20/50, Russia) with mesh sizes of 1, 0.5, and 0.25 mm. During preliminary separations, the 2 mm sieve retained only single fibres, so it was excluded from further analyses. The flushing procedure for each sample required approximately 3 L of water. To retain the finest particles that passed through the last sieve, the water was allowed to settle, the transparent supernatant fluid was poured off, and the remainder was carefully poured on pre-weighed filter paper with a pore size of 3–5 µm (Blue ribbon, Melior XXI, ash content 0.01%, Russia). The particles retained on the sieves were also transferred to pre-weighed filter papers. All particles on filter papers were dried at 80 °C to constant mass, and weighed to 0.01 mg. Particles retained on the 1 mm sieve are referred to as 'large particles', on the 0.5 mm sieves as 'medium particles', on the 0.25 mm sieve as 'small particles', and those that passed the 0.25 mm sieve as 'very fine particles' or 'fines'.

To compare the sizes of digesta particles, mean particle sizes (MPS) were calculated using the DMEAN method as described by Fritz et al. (2012). For this procedure, the amount of dry matter retained per sieve/filter paper (S_i) is expressed as a proportion ($p_{(i)}$) of the total amount retained; this proportion is then multiplied by the size that the respective sieve/paper represents, that is, the mean of the pore size of the sieve/filter paper and the preceding sieve's pore size $[(S_{(i+1)} + S_{(i)})/2]$, and assuming a maximum of 1.5 mm for the largest particles:

$$\mathrm{MPS} = \sum_{i=1}^{n} p(i) * \frac{S(i+1) + S(i)}{2}.$$

This procedure was done with (MPS_{fines}) and without ($MPS_{nofines}$) the residue on the filter paper. Additionally, the proportion of residue on the filter paper (fines) in the total dry matter of the faeces was calculated, as well as the proportion of residue on the 1 mm sieve (large particles) and the 0.25 mm sieve (small particles) of the dry matter on all sieves (i.e., excluding the fines). Because the proportion of medium particles (of large, medium and small particles) makes up the difference to 1, this fraction was not assessed separately. Additionally, the reduction in particle size from glandular stomach to faeces was calculated in absolute (mm) and relative (%) terms, as the difference in MPS, and as

the difference in % of the MPS of the glandular stomach, respectively.

Statistical evaluations used *R* version 3.4.1 (2017). Comparisons of individual measurements among groups were done by ANOVA (confirming normal distribution of residuals) and subsequent Tukey's post hoc tests. Depending on normality, as assessed via the Shapiro–Wilk test, correlations between measurements were examined by Pearson's *R* or Spearman's *rho*. For digesta particle size, a linear mixed effects model employing the lmerTest package (Kuznetsova et al. 2017) was used, with MPS as a dependent variable, diet and GIT compartment as cofactors, individual animal as a random factor to account for repeated measures, and the diet × GIT compartment interaction. Differences among diets and GIT compartments were separated by least squares means difference post hoc test. Normal distribution of residuals was tested by Shapiro–Wilk test. As this was not given for those mixed models where proportions of particles were the dependent variable, these models were repeated using ranked data, and only those results are reported here. Data are presented as means ± SD, and $P < 0.05$ was accepted as the level of significance.

Results

The four chenopod species differed in fibre content and in ash content (Table 1). The final body mass did not differ among the four dietary groups (Table 1; $P = 0.121$). Daily dry matter intake (DMI) differed among the groups (Table 1, $P = 0.001$), ranging from 9.4 ± 1.7 to 13.0 ± 1.2 g/d, and was lower for *Anabasis articulata* than for the other diets ($P = 0.009$). Expressed as relative daily DMI (g/kg$^{0.67}$), there was still a difference among groups (Table 1, $P = 0.003$), but in this case, only between *Anabasis articulata* and *Atriplex halimus* ($P = 0.002$). Apparent dry matter digestibility also differed among groups (Table 1, $P < 0.001$), ranging between 59 ± 3 and 71 ± 1%, and was lowest in the *Anabasis articulata* group. Correspondingly, the relative digestible DMI also differed among groups (Table 1, $P = 0.002$), being again lowest in the *Anabasis articulata* group. Dry matter digestibility correlated negatively with neutral and acid detergent fibre content (NDF: *rho* = -0.87, $P < 0.001$; ADF: *rho* = − 0.83, $P < 0.001$; Fig. 1a), but there was no correlation between body mass and intake ($P = 0.255$), body mass and digestibility ($P = 0.127$), and relative intake and

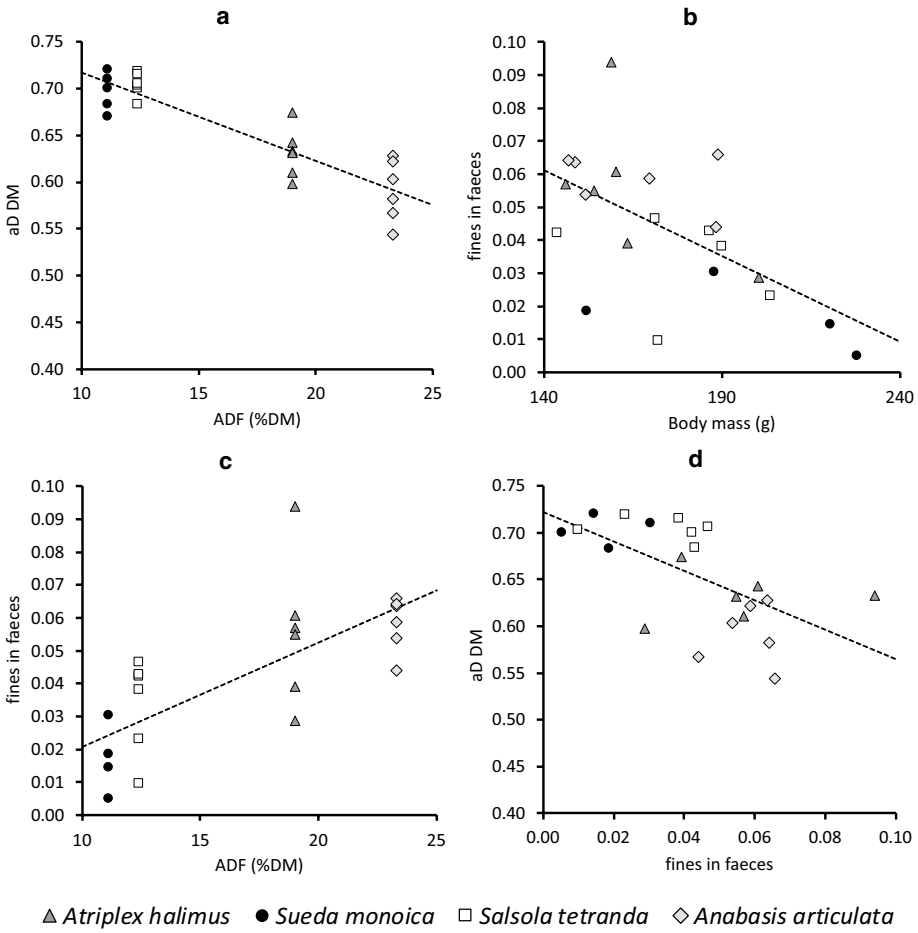

Fig. 1 Correlations between (**a**) acid detergent fibre (ADF, in % dry matter) and apparent digestibility of dry matter (aD DM), (**b**) body mass and the proportion of very fine particles ('fines'; of all particles) in the faeces, (**c**) ADF and fines in the faeces, (**d**) fines in the faeces and aD DM, for four species of chenopods consumed by *Psammomys obesus*. Linear regression models (on diet averages when ADF is involved) [with 95% confidence intervals]: (**a**) aD DM = 0.81 [0.78,0.84]— 0.009 [− 0.011, − 0.007] ADF, $R^2 = 0.97$, $P = 0.011$; (**b**) fines in faeces = 0.13 [0.08,0.19] – 0.001 [− 0.000, − 0.001] BM, $R^2 = 0.32$, $P = 0.004$; (C) fines in faeces = − 0.01 [− 0.04,0.02] + 0.003 [0.002,0.005] ADF, $R^2 = 0.81$, $P = 0.065$; (D) aD DM = 0.72 [0.68,0.76] − 1.57 [− 2.44, − 0.70] fines in faeces, $R^2 = 0.35$, $P = 0.002$

digestibility ($P = 0.277$). Body mass tended to be related negatively to dietary ADF (*rho* = -0.39; $P = 0.064$).

Body mass correlated positively with faecal MPS_{fines} (*rho* = 0.43, $P = 0.047$) but not with faecal $MPS_{nofines}$ ($R = 0.29$, $P = 0.185$); correspondingly, body mass correlated negatively with the proportion of fines in the faeces ($R = -0.60$, $P = 0.003$; Fig. 1b). Body mass did not correlate with the proportion of small ($P = 0.303$) or large ($P = 0.388$) particles in the faeces. Faecal MPS_{fines} was correlated negatively with ADF (*rho* = − 0.48, $P = 0.024$), but was neither correlated with NDF ($P = 0.359$) nor ADL ($P = 0.579$); by contrast, faecal $MPS_{nofines}$ was not correlated with any fibre concentration ($P > 0.132$). Neither faecal MPS_{fines} ($P = 0.150$) nor faecal $MPS_{nofines}$ ($P = 0.698$) was correlated with digestibility. The proportion of fines in the faeces correlated positively with NDF (*rho* = 0.65; $P = 0.001$) and ADF (*rho* = 0.78; $P < 0.001$; Fig. 1c), and negatively with digestibility ($R = -0.62$; $P = 0.002$; Fig. 1d), but digestibility was not correlated with the proportion of large or small particles in the faeces ($P > 0.596$). There was no correlation between the ratio of ADL in NDF and any particle size or digestibility measure ($P > 0.195$) or between the reduction in particle size based on MPS_{fines} ($P = 0.215$) or $MPS_{nofines}$ ($P = 0.736$) in the GIT and digestibility.

MPS_{fines} was smaller in the forestomach than the glandular stomach, and decreased from the small intestine to the hindgut (Table 2, Fig. 2a). The same effect was evident for the proportion of very fine particles (Table 2, Fig. 2e). When these particles were excluded, there was no difference in $MPS_{nofines}$ or the proportion of large or small particles between the forestomach and the glandular stomach (Table 2, Fig. 2b–d). However, even when the very fine particles were excluded, there was a decrease in $MPS_{nofines}$ and the proportion of large particles, and an increase in the proportion of small particles between the small intestine and the hindgut (Table 2, Fig. 2b–d). Diet had a significant effect on particle size distribution, with *Atriplex halimus* and *Suaeda monoica* generally yielding relatively large and *Salsola tetrandra* relatively small particles (Table 2, Fig. 2). The only exception to this pattern was, again, in the very

Table 2 Results of the linear mixed effects models, using diet and gastrointestinal (GIT) compartment as cofactors, and individual as random factor

	F	*P*	Post hoc
Dependent variable: mean particle size (including very fine particles)—MPS_{fines}			
Individual	–	< 0.001	–
Diet	6.95	0.002	*Atripl. halimus* > *Salsola tetrandra*; *Suaeda mon.* > (*S. tetrandra*, *Anab. articulata*)
GIT compartment	32.8	< 0.001	Glandular stomach > (Forestomach, Small intestine) > (Caecum, Colon) > Faeces
Diet × GIT interaction	1.96	0.027	–
Dependent variable: mean particle size (excluding very fine particles)—$MPS_{nofines}$			
Individual	–	< 0.001	–
Diet	10.8	< 0.001	(*Atripl. halimus, Suaeda mon.*) > *Anab. articulata* > *Salsola tetrandra*
GIT compartment	22.2	< 0.001	(Forestomach, Glandular stomach, Small intestine) > (Caecum, Colon) > Faeces; Glandular stomach > Small intestine
Diet × GIT interaction	2.17	0.013	–
Dependent variable: proportion of large particles (ranked)			
Individual	–	< 0.001	–
Diet	11.6	< 0.001	(*Atripl. halimus, Suaeda mon.*) > *Anab. articulata* > *Salsola tetrandra*
GIT compartment	24.8	< 0.001	(Forestomach, Glandular stomach, Small intestine) > (Caecum, Colon) > Faeces
Diet × GIT interaction	2.31	0.008	–
Dependent variable: proportion of small particles (ranked)			
Individual	–	< 0.001	–
Diet	14.3	< 0.001	*Salsola tetrandra* > *Anab. articulata* > (*Atripl. halimus, Suaeda mon.*)
GIT compartment	13.9	< 0.001	(Forestomach, Glandular stomach) < (Small intestine, Caecum) < (Colon, Faeces)
Diet × GIT interaction	1.70	0.064	–
Dependent variable: proportion of very fine particles (ranked)			
Individual	–	< 0.001	–
Diet	9.29	< 0.001	(*Atripl. halimus, Salsola tetrandra, Anab. articulata*) > *Suaeda mon*
GIT compartment	21.7	< 0.001	(Glandular stomach, Small intestine) < Forestomach < (Caecum, Colon, Faeces)
Diet × GIT interaction	3.11	< 0.001	–

Degrees of freedom for diet $F_{3,19}$, for GIT compartment $F_{5,94}$, for the interaction $F_{15,94}$

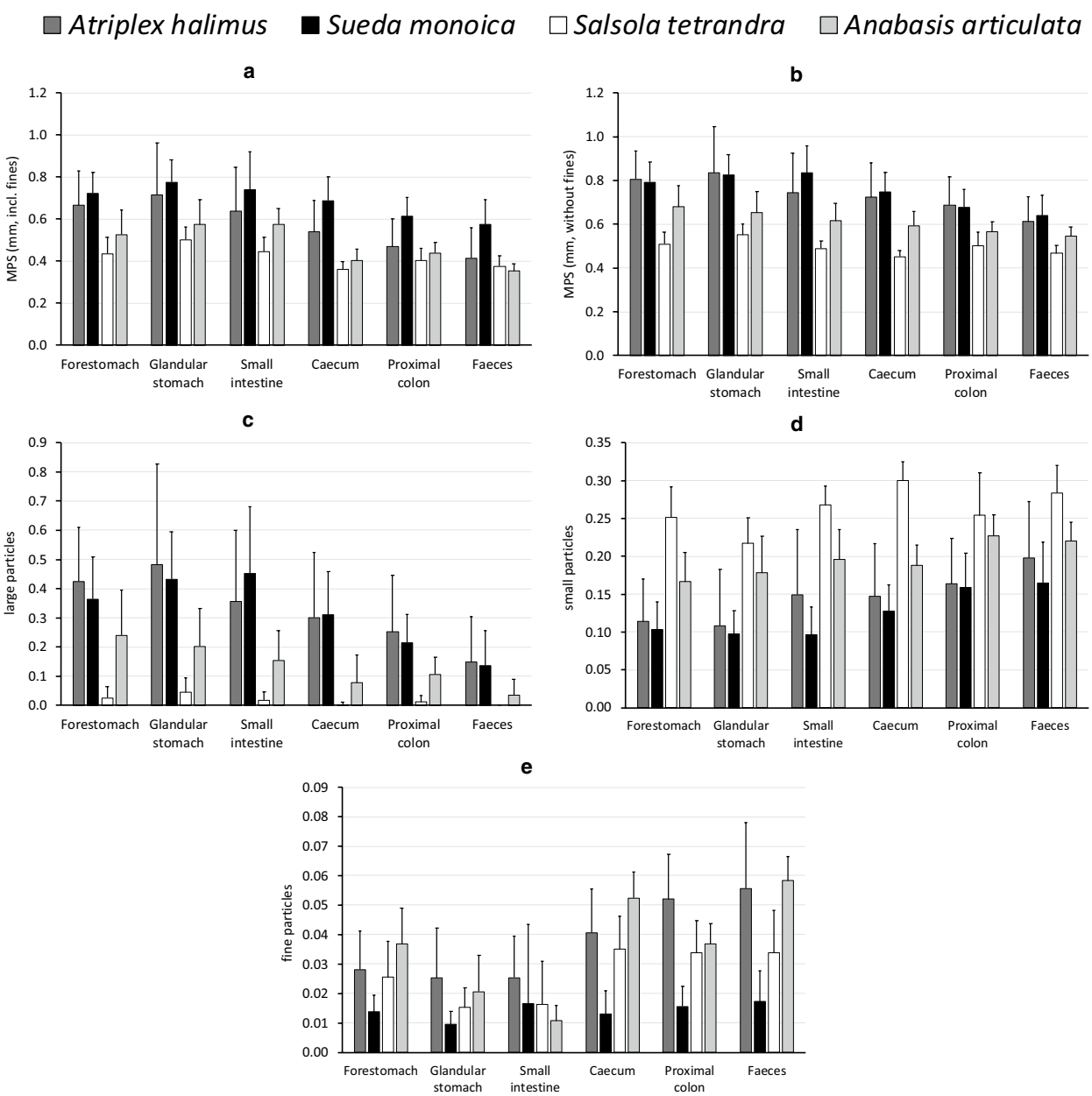

Fig. 2 Distribution along the gastrointestinal tract of (**a**) mean particle size (MPS) calculated including very fine particles (< 0.25 mm, 'fines'), (**b**) MPS calculated without very fine particles, (**c**) the proportion of large particles (> 1 mm; of all particles, excluding fines), (**d**) the proportion of small particles (< 0.5 mm and > 0.25 mm; of all particles, excluding fines), (**e**) the proportion of very fine particles (< 0.25 mm; of all particles), for four species of chenopods consumed by *Psammomys obesus*. Columns indicate means and sd per treatment. For statistics, see Table 2

fine particles, of which *Suaeda monoica* yielded less than the other diets (Table 2, Fig. 2e). The Diet × GIT interaction was generally significant, indicating that the particle size pattern was quantitatively not uniform across diets. Nevertheless, in spite of evident numerical variation, there was no significant difference in the absolute or relative particle size reduction from the material in the glandular stomach to the faeces (Table 1).

Discussion

The present study confirmed our first prediction that mean particle size decreases along the gastrointestinal tract of terrestrial herbivores, and that this reduction is not just an artefact due to the inclusion of putative microbes in the particle size calculation. Concomitantly, there was support for our second prediction that the proportion of very fine particles,

putatively microbes, increases at sites of presumably higher microbial activity (the forestomach and the hindgut). Unfortunately, a more detailed analysis of the different particle size fractions, e.g., by chemical analysis for crude nutrients or microbial markers such as diaminopimelic acid (Siddons et al. 1982), or by microscopic methods, was beyond the logistical scope of our study. In future studies, detailed differentiation between dietary and microbial particles would be interesting. Possibly, visual investigations of the digesta particles from different GIT sections (Nørgaard et al. 2004) and electron microscopy would be required to yield insight into the reduction of particle size due to microbial action.

The fraction of digesta passing the finest sieve can, at times, represent more than 50% of all material retrieved from faeces or a certain compartment (Matsuda et al. 2014; Naumova et al 2017). We caution against equating this fraction as being only food-derived particles, and recommend that in the absence of more detailed investigations of this fraction, particle size distribution should be evaluated both with and without this fraction, as was done in the present study. For example, the present study could lead to the conclusion that faecal MPS_{fines} increased with body mass, thus corroborating a general inter-specific finding (see Introduction) that is typically not matched intra-specifically (Clauss et al. 2015). However, there was no relationship between faecal particle size and body mass when the former was expressed as $MPS_{nofines}$, i.e. without the very fine particles. This indicates that in our study, animals of lower body mass only had a higher proportion of very fine particles in their faeces (Fig. 1b), but not generally larger particles originating from the diet.

With respect to a colonic separation mechanism (CSM), we had expected an accumulation of very fine particles in the caecum of this small herbivore (Lanyon and Sanson 1986; Vispo and Hume 1995; Naumova et al. 2015a, b, 2017; Clauss et al. 2019), because fat sand rats have both the morphological and the behavioural correlates of a CSM (see Introduction). However, this expectation was not supported. We can only speculate that this was due, at least in part, to the time lag between the last coprophagic event and sampling, which possibly varied among animals (Naumova et al. 2015a, b), even though all animals were euthanized at the same time. In future studies, a detailed recording of coprophagic events is recommended. With respect to the rodent forestomach, the increased proportion of very fine particles in the present study indicates that microbes are active at this site. The lower proportion of very fine particles in the glandular stomach and small intestine suggests that these microbes, once passed on, are disintegrated by enzymatic digestion at these sites.

As in other studies (Renecker and Hudson 1990; Hummel et al. 2008; Jalali et al. 2012a, b, 2015; Kljak et al. 2019), we found the predicted effect of diet on digesta particle size. This raises the question about the plant factors that influence chewing efficiency and microbial particle size reduction. When the very fine particles were excluded, no relationship was observed between $MPS_{nofines}$ and any fibre fraction. Differences may be related to variation in ash content among the diets, the high oxalate content in some chenopod species, or differences in secondary compounds (Palgi et al. 2005, 2008) that may make digesta particles resistant to microbial action to varying degrees. Similar to a previous study in domestic herbivores (Jalali et al. 2012a), this study does not support the intuitive yet simplistic concept that a more digestible diet should result in smaller digesta particles. Even though the diet with highest digestibility, *Salsola tetrandra*, was generally comminuted into the smallest digesta particles, the diet with the largest digesta particles, *Suaeda monoica*, was not the least digestible. Digesta particle size can be considered a proxy for comminution processes such as chewing efficiency, but not as a proxy for nutritive quality or digestibility of a diet. Thus, when comparing chewing efficiency across species, the possible influence of intraspecific variation due to dietary selection should always be taken into account (Hummel et al. 2008), as diet also has an effect on particle size.

As typical for herbivores of any size (Demment and Van Soest 1985; Hagen et al. 2015), we observed a negative relationship between dietary fibre and digestibility (Fig. 1a). While this finding was not surprising, the observation that less digestible diets led to an increase of very fine particles, i.e., putatively microbial particles, in the faeces (Fig. 1d), does not correspond to current understanding of the presence of microbial protein in the faeces of herbivores. Actually, an increase in faecal nitrogen and, in particular, metabolic (i.e., mainly microbial) faecal nitrogen in faeces is well established as an indication of an increased, not a decreased diet digestibility in ruminants and horses (Mésochina et al. 1998; Lukas et al. 2005; Clauss et al. 2015; Gálvez-Cerón et al. 2015). Additionally, we would expect the CSM to prevent high proportions of microbes being excreted in the hard faeces (Bjornhag and Snipes 1999). Whether faecal nitrogen or metabolic faecal nitrogen function as digestibility proxies in coprophageous rodents remains to be investigated. Our results suggest that across natural diets, those higher in fibre content trigger a greater loss of very fine particles and, hence, microbial matter with the faeces. This was linked not only to dietary fibre and digestibility, but also to the body mass at the end of the feeding period.

Conclusions

Post-chewing reduction in digesta particle size along the digestive tract of the fat sand rats amounted to 15–25% of the initial value. This size reduction must have occurred

as a consequence of the action of digestive enzymes and microbes. Given previous reports on large herbivores, the hypothesis arises that this may be particularly evident in small herbivores with already small digesta particles after mastication. However, in accord with previous findings, we show that the degree of particle size reduction is not a function of diet quality and digestibility. Hence, digesta or faecal particle size cannot be considered a proxy for diet quality, and particle size reduction within the gastrointestinal tract cannot be considered only an adaptation for optimizing digestion. Rather, it is a side effect of digestion, the determinants of which—with respect to the physical or chemical plant factors controlling its degree—are still unknown. Although our findings are in agreement with the sparse literature on particle size analysis, we must remain cautious as to whether they can be transferred to other small mammals that ingest diets that are more varied than those of fat sand rats.

When analysing particle size in digesta or faeces, it is important to consider that very fine particles, typically those not retained on sieves, may not only represent extremely comminuted food, but also microbes: fractions < 0.15 mm may contain protozoa (Dehority 1993), and fractions < 0.01 mm may contain bacteria (Frobisher et al. 1974). If these fractions are included in the calculation of mean particle size, gastrointestinal compartments that harbour microbes may, at similar food particle size, appear to contain smaller overall particle sizes. Further studies on the fractionation of this class of very fine particles, and in vitro studies of the effect of microbial digestion on the size of particles of varying initial sizes, are required to fully understand the relevance of this digesta component.

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Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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